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Preliminary

A Role of Formate Dehydrogenase in the Oxalate Metabolism in the Wood-destroying Basidiomycete *Ceriporiopsis subvermispora*[*1]

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Introduction

It is a common physiological trait that brown-rot fungi, including *Fomitopsis palustris* accumulate oxalic acid in large quantities in the cultures. Oxalic acid serves as an acid catalyst for the hydrolytic breakdown of wood polysaccharides during brown-rot wood decay processes. Furthermore, *F. palustris* has been reported to acquire energy for growth by "oxalate-fermentation"[1]. An oxalate-producing enzyme, glyoxylate dehydrogenase (GLOXDH) linked with oxalate biosynthesis, and isocitrate lyase (ICL) as a key enzyme of the glyoxylate cycle, have been successfully purified and characterized from *F. palustris*[2,3]. Furthermore, another oxalate-producing enzyme, oxaloacetase has been detected from wood-rotting fungi[4].

On the contrary, white-rot fungi accumulate much smaller amounts of oxalic acid because they have oxalate-decomposing systems[5–8]. Under the extracellular condition, the two biochemical mediators, including veratryl alcohol cation radicals and Mn^{3+} produced by lignin peroxidase and manganese peroxidase, respectively, have been reported to catalyze the decomposition of oxalic acid to carbon dioxide[9,10]. As a result, oxalic acid seemingly inhibits ligninolytic enzymes[4]. Furthermore, it has been proposed as a general mechanism for intracellular oxalate metabolism that oxalate decarboxylase (ODC ; EC 4.1.1.2) converts oxalate to formate and carbon dioxide, and the formate thus produced is converted to carbon dioxide by formate dehydrogenase (FDH ; EC 1.2.1.2), yielding NADH. However, recently, Aguilar *et al.* successfully purified oxalate oxidase (OXO ; EC 1.2.3.4) from white-rot fungus *Ceriporiopsis subvermispora* and they proposed a novel pathway in which oxalate is metabolized by OXO to carbon dioxide, accompanied with the production of H_2O_2[11].

Thus, we were motivated to investigate whether *C. subvermispora* has the oxalate-metabolizing systems with ODC, FDH and OXO[11]. We report here preliminary results for the purification and characterization of FDH and the detection of ODC activity from *C. subvermispora*. The results are discussed in relation to oxalate metabolism by this fungus.

Results and Discussion

Ceriporiopsis subvermispora CS105 that was kindly provided from Dr. Vicuña was cultivated at 27°C in the Kirk's basal medium[12] containing 2.5% glucose as a carbon source, 3.0 mM ammonium tartrate as a nitrogen source, which was supplemented with 7-fold minerals. We have purified FDH from *C. subvermispora* by various column

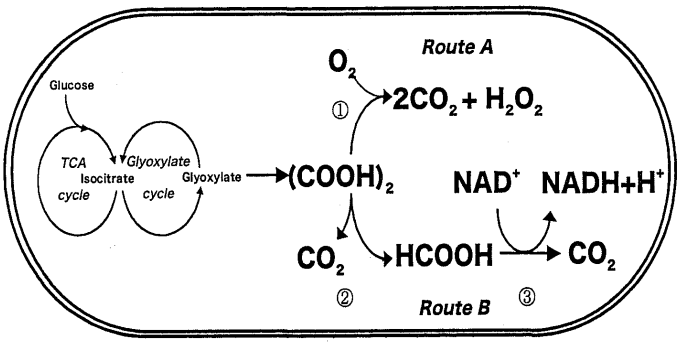

Figure A possible biochemical mechanism for oxalate metabolism in *C. subvermispora*. Notes : ① Oxalate oxidase, ② Oxalate decarboxylase, ③ Formate dehydrogenase.

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chromatographies. The purified FDH was found to be electrophoretically a single band on SDS-PAGE gel. The purified FDH was similar in molecular mass to the FDHs purified from yeasts[13] and plants[14]. But the K_m value for formate was about one twentieth that of the yeast enzyme, although the K_m value for NAD^+ was almost the same[13]. The enzyme showed greater activities at the neutral pH range.

The optimum temperature for native FDH was at a room temperature. Formate was the best substrate among various intermediate organic acids tested. The FDH activity was inhibited by NADH (60 μM), ATP (10 mM), and ADP (10 mM). Interestingly, 2-oxoglutarate and oxaloacetate also inhibited the enzymes. These results suggest that these α-ketoacids may control the enzyme activity intracellularly.

The ODC activity was detected from the cell-free extracts of *C. subvermispora*. Thus, the results strongly suggest that *C. subvermispora* decomposes oxalate to CO_2 via formate (Figure, Route B), besides another oxalate metabolizing pathway which was reported by Aguilar *et al.* (Route A)[11].

We suspect that NADH produced as the results of the oxidation of formate may serve as an electron donor for ATP generation as in the case of yeasts[15]. Alternatively, NADH may be used as a cosubstrate for several enzymes to reduce quinones derived from lignin. It is speculated that white-rot fungi are superior to brown-rot ones in biochemical evolution on conversion of oxalate to an energy source. However, further research is needed to elucidate the reaction mechanisms for oxalate metabolism and the role in white-rot wood decay (Figure).

References

1) E. Munir, J.J. Yoon, T. Tokimatsu, T. Hattori and M. Shimada: *Proc. Natl. Acad. Sci. USA.*, **98**(20), 11126–11130 (2001).
2) E. Munir, T. Hattori and M. Shimada: *Arch. Biochem. Biophys.*, **399**(2), 225–231 (2002).
3) T. Tokimatsu, Y. Nagai, T. Hattori and M. Shimada: *FEBS Lett.*, **437**(1–2), 117–121 (1998).
4) Y. Akamatsu, M. Takahashi and M. Shimada: *Mokuzai Gakkaishi*, **39**, 352–356 (1993).
5) H. Shimazono: *J. Biolchem.*, **42**, 321–340 (1955).
6) H. Shimazono: *Bull. Forest Expt. Station*, **33**, 393–397 (1951).
7) S. Takao: *Appl. Microbiol.*, **13**, 727–732 (1965).
8) M.V. Dutton, C.S. Evans, P.T. Atkey and D.A. Wood: *Appl. Microbiol. Biotechnol.*, **39**(1), 5–10 (1993).
9) Y. Akamatsu, D.B. Ma, T. Higuchi and M. Shimada: *FEBS Lett.*, **269**, 261–263 (1990).
10) D.B. Ma, T. Hattori, Y. Akamatsu, M. Adachi and M. Shimada: *Biosci. Biochem. Biotechnol.*, 56, 1378–1381 (1992).
11) C. Aguilar, U. Urzua, C. Koening and R. Vicuna: *Arch. Biochem. Biophys.*, **366**, 275–282 (1999).
12) T.K. Kirk, E. Schultz, W.J. Connors, L.F. Lorenz and J.G. Zeicus: *Arch. Microbiol.*, **117**, 277–285 (1978).
13) T.V. Avilova, O.A. Egorova, L.S. Loanesyan and A.M. Egorov: *Eur. J. Biochem.*, **152**, 657–662 (1985).
14) D. Peacock and D. Boulter: *Biochem. J.*, **120**, 763–769 (1970).
15) N. Kato, H. Sahm and F. Waner: *Biochim. Biophys. Acta*, **566**(1), 12–20 (1979).